complex formation (Wrana, J. 1998. *Miner. Electrolyte Metab.* 24:120-130). It is now well established that TGF-β signaling pathways switch target genes on through the activities of Smad proteins. These cytosolic proteins are recruited and phosphorylated by the TGF-β, activin, or BMP receptor complexes. Smad proteins exist as monomers in unstimulated cells but homo- or hetero-dimerize and translocate to the nucleus of the cells where they then activate target gene expression through contact with cofactors and DNA.

Please amend the second paragraph beginning on page 2, line 16, as follows:

Thus, although much is now known about how TGF-β pathways switch genes on, little is known about how genes can be switched off. There are examples of such negative regulation in vertebrates and in model organisms such as *C. elegans* and *Drosophila*. In mammals, growth inhibition by TGF-β is correlated with negative regulation of *c-myc* and *cyclin A* (Feng, X.H. et al. 1995. *J. Biol. Chem.* 270:24237-24245). TGF-β also negatively regulates proteases that degrade components of the extracellular matrix such as collagen (Kerr, L.D. et al. 1990. *Cell* 61:267-278). Evidence that Smad proteins can directly repress or

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86:401-409; Theisen, H. et al. 1996. *Development* 122:3939-3948; Tomoyasu, Y. et al. 1998. *Development* 125:4215-4224; Chanut, F. and U. Heberlein. 1997. *Development* 124:559-567).

Please amend the paragraph on page 3, beginning on line 20, as follows:

Although repression by TGF-β pathways could be indirect, mounting evidence shows that Smad proteins interact directly with a variety of co-repressors. Smad proteins interact with the repressors Evi-1 (Kurokawa, M. Et al. 1998. *Nature* 394:92-96), Gli3 (Liu, F. Et al. 1998. *Nature Genet.* 20:325-326), TGIF (Wotton, D. Et al. 1999. *Cell* 97:29-39), SIP1 (Verschueren, K. Et al. 1999. *J. Biol. Chem.* 274:20489-20498), and the oncoproteins SKI (Luo, K.S. et al. 1999. *Genes Develop.* 13:2196-2206), SnoN (Stroschein, S.L. et al. 1999. *Science* 286:771-774), and adenovirus E1A (Nishihara, A. et al. 1999. *J. Biol. Chem.* 274:28716-28723). It is known that some viruses inhibit cellular responses to TGF-β and the finding that E1A interacts directly with Smad proteins supports this finding. Binding of Smad3 to E1A or TGIF inhibits Smad binding of the coactivator p300. Contact with TGIF or SKI recruits histone deacetylase and inhibits transcriptional activation by Smad2 and Smad3. Contact

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negatively regulate transcription comes from genetic analysis of the *C. elegans* TGF-β pathway that regulates choice between reproductive growth and diapause (Patterson, G.I. et al. 1997. *Genes Develop.* 11:2679-2690). Activation of this pathway over-rides negative regulation by the Smad4-related Daf-3 protein. Negative regulation by Smad proteins was also shown in *Drosophila*, where the *Drosophila* BMP4 homolog, decapentaplegic (dpp), was shown to activate its targets by repressing expression of a novel repressor known as Brinker (Campbell, G. And A. Tomlinson. 1999. *Cell* 96:553-562; Jazwinska, A. Et al. 1999. *Cell* 96:563-573; Minami, M. Et al. 1999. *Nature* 398:242-246; Sivasankaran, R. Et al. 2000. *EMBO J.* 19:6162-6172). Ectopically expressed Brinker was able to repress BMP targets in frog embryos as well, indicating that this double negative mechanism is likely to operate in vertebrates as well as in *Drosophila*. A second negatively regulated target is the segment polarity gene, wingless (wg), which is repressed in response to Dpp in the embryonic ectoderm (Grieder, N. et al. 1995. *Cell* 81:791-800) and in imaginal discs (Penton, A. and F.M. Hoffmann. 1996. *Nature* 382:162-165; Brook, W.J. and S.M. Cohen. 1996. *Science* 273:1373-1376; Jiang, J. and G. Struhl. 1996. *Cell*

with Evi-1 inhibits DNA binding of Smad3. However, because Smad proteins are not known to have any intrinsic ability to function as repressors, and in fact have just the opposite effect, the function generally ascribed to DNA-binding Smad co-repressors is one of dampening of transcriptional activation by Smads, leaving the mechanism of TGFβ-induced repression unexplained. Until the present invention it was not appreciated that Smad proteins are able to interact directly with co-repressor genes through a specific Smad domain. Thus, the present invention describes the interaction between Smad proteins and the general co-repressor dCtBP and shows how this interaction provides a mechanism for the ability of activated Smads to directly repress transcription in response to signaling.

A3 Cont'd

Please amend the paragraph beginning on page 6, line 10, as follows:

Figure 3 depicts the results of experiments examining the ability of dCtBP to inhibit activation of Smad box-lacZ reporter by Mad and Medea in Drosophila cells. The data shown are the average of three S2 transfections. Each LRR repeat contained three Smad boxes arranged as : AGAC GTCT GTCT (SEQ ID NO:1).

A4